Spekwin32

Complete Documentation

software: version 1.72
documentation: version 3.2

TABLE OF CONTENT

1. General Description

1.1. Development History

The original software named Spekwin (16bit version) was developed in the Daltrozzo group at the Universität Konstanz in 1997-1998 by Dr. Claus Vielsack with DELPHI1. It was based on an even earlier attempt to create software for spectra display undertaken by Dr. G. Kollmannsberger with TURBO PASCAL (DOS program tpbld.exe, until 1994). Since 1999, further development is done by me. At present there is the 32bit-Version **Spekwin32** (developed with DELPHI 4) with enhanced functionality and much increased working speed for some functions. A big part of the work took place during my PhD time at the Daltrozzo group from 2000-2005. Development speed went down somewhat afterwards. Since 2010, many new functions have been introduced.

1.2. Description

One main goal of Spekwin32 is the consistent display of spectra coming from different sources. At the moment, about 36 different spectral file types from commercially available spectrometer systems can be read. By using Copy&Paste via the clipboard, arbitrary data can also be introduced into Spekwin32 for fast visualization. Unknown binary files can converted into ASCII data for a first attempt of reading their content. Spekwin32 is able to handle UV/VIS, fluorescence, NIR, IR and Raman spectra while the main focus is on UV/VIS and fluorescence.

Spekwin32 allows doing many tasks on (series of) spectra that come across in the laboratory everyday life (baseline correction, spike removal, normalization, smoothing, averaging, integration, finding peaks and determining FWHM, showing peak labels, calculation of concentration via Lambert-Beer, absorption coefficient and oscillatory strength, create Raman shift spectra ...).

As important features for fluorescence spectroscopy there are: calculation of degree of polarization, center of gravity, fluorescence quantum yield; correction of excitation intensity of fluorescence spectra, calculation of excitation spectra from two-dimensional fluorescence spectra, removal of Rayleigh and Raman peaks from EEM data, subtraction of Blank EEM data, displaying two-dimensional fluorescence spectra (EEM) from series of fluorescence or excitation spectra. The EEM functions are useful for pretreatment of data intended for chemometric analysis. To simulate absorbance spectra, there is an algorithm that allows fitting of any group of Gauss curves to present spectra.

For simple documentation, any number of spectra can be saved together as proprietary Spekwin32 binary file or as two sorts of human readable ASCII files (*.dat and *.csv). Spectral data can also be exported into two commonly accepted spectra file formats: JCAMP-DX and THERMO Galactic/GRAMS spc files. A batch export function allows to convert hundreds of spectra from all file formats into single spectrum *.dx, *-spc and *.csv files. Gemologists will like

the possibility to export Raman spectral data into RRUFF format. The current plot can be printed, copied to the clipboard or saved as graphics (WMF, GIF, TIFF, BMP, and PNG). For LaTeX freaks, Gnuplot parameter files and data files can be created that allow producing EPS files directly. The current's plot spectral data can also be copy&pasted via the clipboard.

1.3. System Requirements/ Installation

Spekwin32 will work with Win XP/Vista/7/ 8.1/ 10. Hardware requirements are minimal. The downloaded file will install the full english version, including the HTML manual. There is also a german version available. Please run the installation in administrator mode, otherwise reading of *.spc files will not work.

1.4. Terms of Use

Spekwin32 is provided as freeware for non-commercial, private, academic and educational use, this includes also non-profit organization (i.e. schools, universities, UN organizations, public authorities, police, fire brigade, hospitals). In this case, you are granted the right to use and to make an unlimited number of copies of this software.

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1.5. Scientific citations

If you use Spekwin32 in your scientific work, please reference to this software as:

F. Menges "Spekwin32 - optical spectroscopy software", Version 1.72.0, 2015, http://www.effemm2.de/spekwin/

Please insert the current version number and the year of compilation from the *Help => about Spekwin32...* window. You may leave out the part in quotation marks.

(And please: try to get the spelling right! It's not "Specwin-32" and not "Speckwin 32", there is no blank and no hyphen between "Spekwin" and "32", just write **Spekwin32**!)

2. Main Window and Main Menu

2.1. Main Menu

All functions (except Zoom/Rescale) are accessed via the Main Menu.

2.2. Icon Bar

Some of the more important functions can also be accessed via the icons of the Icon Bar. The function of icons is shown with the mouse pointer shortly hovering above them.

2.3. Graph Window

The Graph Window is always visible, consisting of axes with axis titles, loaded spectra and legend with corresponding legend texts. As x axis types, there can be wavelength, wavenumbers, Raman Shift and Electron Volts; as y axis types, there is transmittance (or reflectance), absorbance (or intensity), epsilon or log(epsilon) available. When no spectrum is loaded, there are no axis titles.

 Axes might be changed in *[Plot/Options]→[Axes]*. The font of **axis ticks** might be changed in *[Plot/Options]→[Axes Font]*, the font of axis titles and **legend titles** might be changed together in *[Plot/Options]→[Legend Font]*. These might also be permanently changed in *[Plot/Options]→ [Configuration]*. The properties of spectra display (colour, line type, line width) and the corresponding text of the legend title can be changed in *[Spectra]/[General]*.

The mouse buttons are for **zooming** in and out. Select the zoom range with the left mouse button. The program then zooms in to the next axis ticks for a straight axis numbering. The middle mouse button redraws the graph window. The right mouse button does a rescale, all spectra will be displayed completely.

The legend box can be moved with the left mouse button. It might be hidden with *[Plot/Options]→[Legend on/off]*.

2.4. Status Bar

The Status Bar at the lower edge is divided in three parts:

- On the left, the current number of loaded spectra is shown.

- On the middle, various program messages will be shown.

- On the right, the current mouse position (x axis | y axis) is continuously shown in units of the graph axes.

3. The FILE Menu

The File menu contains all functions to Open, Save, Print and Export spectra. At the bottom you will find a list of the most recently used Spekwin32 files (only *.spv files).

3.1. Menu Item [Open]

Several files can be selected simultaneously in the usual manner. While loading the selected spectral files, the status of the reading procedure is displayed in the middle part of the status bar. If possible, the x and y axis types are chosen according to the most recently opened spectral file. The following file formats are currently recognized (you will find a collection of sample files at the Spekwin32 website):

*.abs	ASCII file format of StellarNet's SpectraWiz software. File extensions explained:
*.trm	.abs - Absorbance
*.ssm	.trm - Transmittance
*.irr	.ssm - Scope Mode
*.lib	.irr - Irradiance Mode (could be any unit from Watts to Candela)
	.lib - a library file for LIBS and Raman

*.asc :	• ASCII file format of absorption spectra of the Milton Roy MR3000 diode array absorption spectrometer. The y axis type of the file is detected automatically. • ASCII file format of absorbance spectra of the Beckman Coulter DU 600/7000 absorption spectrometers. • ASCII file format of USGS spectral data base.

*.csv :	ASCII file format (csv = comma separated values) of spectra from different sources: • Absorbance spectra of the Varian Cary 50 absorption spectrometer (Software: Cary WinUV). Spectra in transmittance and absorbance will be read. • Absorbance spectra of the Hewlett Packard 8453 absorption spectrometer. • IR spectra of Bio-Rad FTS 3000 MX spectrometer (software: Varian Resolution Pro) • Absorbance spectra of Scinco Neosys 2000 spectrometer (Software: Lab Pro Duo) • Absorbance spectra of WTW photoLab spectral software • Absorbance spectra of the Thermo Electron Helios Alpha spectrometer • multicolumn (1 – many), multiformat (all spectral types) csv file, produced by Spekwin32 itself

*.dat :	Spekwin32 ASCII file format. Contains any number of spectra together with their legend title. The program always takes the y values as absorbance, so be careful if you saved spectra in a different y scale type.

*.dsp	Binary file format of spectra from Ascanis Lambda-SPX/ VISIONlite software

*.dx :	JCAMP-DX 4.24/5.00 –file format of UV/VIS, Raman and IR spectra. NMR and MS spectra will not be read!! DIFDUP and ASDF coded spectra will not be read either. Tested Perkin Elmer software: UVCSS & FLDM (DOS software), UV-Winlab & FL-Winlab. Further Test spectra were available from Bio-Rad DigiLab, Mattson Instruments, Galactic Industries Lab Calc and GRAMS, Sadtler, Jasco HPLC System, NIST Chemistry WebBook, the IUPAC JCAMP site and Robert J. Lancashire's JCAMP site.
*.fak :	Simple ASCII file format for import of your own data. Tab separated XY Pairs, one pair per line, point or comma as decimal separator, optional header: if the first line reads *wavenumbers* or *1/cm* or *cm^-1* or *cm-1* the x axis type is assumed to be wavenumbers, default is wavelength in nm. Also tries to detect y axis type. Automatic query dialog for axis types if unclear from file.
*.ggg :	File format of one of our home built spectrometers.
*.jws	Binary file format from JASCO spectrometers.
*.prn :	• ASCII file format of Princeton Instruments WinSpec 1.6 (16Bit) software for CCD cameras from Princeton Instruments. Save in Winspec as ASCII-1 or ASCII-XY. • ASCII export file format of Horiba DataMax software.
*.proc spec	Binary file format from Ocean Optics SpectraSuite software, internally the format is called "OOIBinary"
*. rruff	ASCII file format of Raman spectra from the RRUFF online mineral database.
*.scn	Binary file format of absorbance spectra of the Beckman Coulter DU 600/7000 absorption spectrometer.s.
*.sp :	Binary and ASCII file formats of absorbance, fluorescence, IR & Raman spectra of PERKIN ELMER spectrometers. Tested Software: UVCSS (DOS software), CFS (DOS software for LS-3B, LS-4B and LS-5B), UV WinLab, FL WinLab. Perkin Elmer Raman test spectra were available from S. B. Engelsen's Database on Raman Spectra of Carbohydrates. Fluorescence spectras' ordinate values get divided by 250 before they are displayed.
*.spc :	• Binary file format from SHIMADZU UV-1600 and UV-1800 spectrometers • Thermo Fisher's binary spectral file format (originating from *Galactic Industries Corporation*). Software: GRAMS/AI. Quasi standard used by quite a lot of other vendors (e. g. Ocean Optics, Jobin Yvon Horiba). Tested spectral types: UV/VIS, NIR, FTIR, Raman, fluorescence. Will **not** read spc files from EDAX XRF analyzers, this is a different file format. For those files, use the "EDAX Spectrum Viewer".

*.spe **:**	Binary file format of Roper Scientific / Princeton Instruments WinSpec/WinView Software (16bit and 32bit). Possible x axis types are wavelength, absolute and relative wavenumbers (for Raman spectra).
*.spk	ASCII file format of absorbance spectra of the IKS XDAP diode array absorption spectrometer (IKS is now axeon in Oberhausen/Germany).
*.spv **:**	Spekwin32's own binary file format. Contains arbitrary numbers of spectra. Legend titles and other properties of individual spectra are saved. Changed file format in versions 1.68.1 and 1.69.2! Earlier program versions are not able to read later *.spv spectral files.
*.trt ***.tat** ***.ttt** ***.tit**	ASCII file format of the Avantes software. Tested: Avantes SpectraWin Basic 5.0 and Avantes Avasoft 6.1. Measuring modes are: trt: "Scope" tat: "Absorbance" ttt: "Transmittance" tit: "Irradiance"
*.wls	ASCII format of VWR UV-1600PC spectrometers, software: "M.wave professional"

3.2. Menu Item [Open unknown binary file]

This menu item will assist you in getting out spectral information from binary files with unknown data formats. After opening your unkown file, a pre-formatted Excel template file opens. The unknown file's content has been put to the clipboard. Please paste this to cell A1 of the Excel template. The first line shows your file's name and filepath. The columns show the file content "as-if" the data was in a certain number format (as there are integer and float types coming with different byte sizes and Endianness). Useful content could show up in any column, normally block-wise. By conditional formatting, the template highlights datablocks with useful numbers.

3.3. Menu Item [Paste Data from Clipboard]

With this feature, you can transfer arbitrary data from Excel or Origin or even any text editor into Spekwin32. It is only required that there are at least two columns, where at least one contains the x axis values. Just select the desired range of data, and copy&paste into Spekwin32. It will work with multiple columns, "x y y y" and "x y x y x y" arrangements are accepted. Axis types can be defined in the line above the data columns. Default types are "wavelength" and "absorbance", if no other information is detected. Optionally, there can be names for the spectra in the line above the axis type descriptions.

Hint: Within Excel, use the keyboard shortcut CTRL+SHIFT+Arrow(down, or right) for automated selection of whole data blocks. Do not select completely empty columns.

3.4. Menu Item [Save]

This menu item will save the current content of the graph window. The graph will be saved with the file name and file type from the last use of *[Save as...]*. On first use, "Save as..." dialog will appear.

3.5. Menu Item [Save Data as]

Used for saving the data of currently displayed spectra. A *"Save as..."* dialog appears for file name, type and path.

There are 5 data file formats to select from:

- **spv** files for fast and space-saving storage and reading in again of spectra in any composition.

- **spc** files for saving of single spectra in Thermo Fisher's quite common file format.

- **dx** file for saving single spectra in the world-wide used, IUPAC-proposed standard file format JCAMP-DX.

- **dat** files for import of values into graphing programs like Origin or Sigmaplot. Multiple spectra get interpolated onto a single common x column.

- **csv** fore easy export of the original spectral data for Excel and the like. Multiple spectra contained as separately xy paired columns.

3.6. Menu Item [Save for RRUFF]

For saving Raman spectra into the native data file format for the RRUFF minerals database (http://rruff.info/about/about_download.php). Their software CrystalSleuth allows to create and maintain your own searchable minerals Raman spectra database.

Select the spectrum to be exported in the upper dropdown list, and use either the parameters from the input file or else put them in yourself. The *[Save]* button will save the selected spectrum in *.rruff format. Quit the window with the *[Close]* button when finished.

3.7. Menu Item [Batch Export Data]

This feature allows to export all loaded spectra as single spectrum files in three file formats (*.spc, *.dx, *.csv). Additionally, all spectra can be exported together as multi-file (same file formats).

- *export data as* individual single spectrum files or else as common multi-file.

- *file location*: choose to save the files in the same location (the filepathes where the original spectrum files were located), or else together at the filepath defined in the edit box below.

- A *new file name* can be defined together with attached successive numbering, or else the legend texts can be used as filenames. Both options get combined, when selecting both.

The [Export!] button starts the file export process; the progress will be shown in the text field and with a progress bar below.

3.8. Menu Item [Save Plot as]

Used for saving current spectra as spectral plot. A *"Save as..."* dialog appears for file name, type and path.

There are 5 graphics file formats to select from:

- **csv** fore easy export of the original spectral data for Excel and the like. Multiple spectra contained as separately xy paired columns.

- **gif** files for saving as internet usable pixel graphics, compressed files, 256 colours, resolution: 1350x900 pixels.

- **bmp** files for saving as lossless pixel graphics, big files, resolution: 1024x768 pixels.

- **wmf** files for saving as freely scalable vector graphics of small file size, recommended best use for Windows Office applications like MS Word or MS PowerPoint.

- **tif** files for saving as rather small lossless compressed files with 32bit colours, best for printing, resolution: 1350x900 Pixel.

- **png** files for saving as small lossless compressed files, the internet picture format of the future, resolution: 1350x900 Pixel.

Hints for optimized graphics export:

- With high resolution screen and maximized program window, the standard axes and legend fonts might look rather small in saved graphics. Try saving again with mid-sized Spekwin32 window.

3.9. Menu Item [Save for Gnuplot]

Creates two files with the same name: a Gnuplot parameter file (*.plt) and a second file (*.tat) with the spectral values. The parameter file contains the necessary commands for Plot Title, Axes titling and scaling, second X-Axes, second Y-Axes (if degree of polarization is included), position of the legend box and **creation of EPS file** with the same name. Before saving, there is a dialog for configuration of these parameters where additional commands can also be included.

For this function the following parameter files must exist in the program folder : *gexit.plt*, *ginit.plt*, *ginit2.plt*, *ginit2d.plt*, *pola36.plt*, *polaset.plt*, *postcol.plt*, *postmon.plt*, *wlachse.plt*, *wlachse2.plt*. This function was tested with Gnuplot 4.6 for Windows (32bit).

3.10. Menu Item [Copy Data to Clipboard]

Copy all data from all current spectra to the clipboard. From there, it can be pasted into text processing or spreadsheet software (like MS Excel or Origin).

Spectral data will be saved in x..y..x..y..x..y.. format (each spectrum has its own paired xy values). In case all spectra have the same x values, the format will be x..y..y..y.. (that is: one x column for all spectra). The first line contains the legend texts, second line contains x and y format for each spectrum.

3.11. Menu Item [Copy Graphics to Clipboard]

The current graph window is copied as a vector graphic to the clipboard. From there, it can be pasted into graphic or text processing software. For optimized graphics see hints in chapter 3.5.

3.12. Menu Item [Print]

Prints the current graph window. Select installed printers like usual. For optimized graphics see hints in chapter 3.5. PostScript files (*.ps) can be produced by printing to a file with a PostScript printer driver.

3.13. Menu Item [Exit]

Terminates the program. Unsaved spectra are lost!

The SPECTRA Menu

3.14. Menu Item [General]

Here you can change all parameters important for the display of spectra. Further, the absorption path length can be set and the sample concentration can be calculated from molecular weight, sample weight and solvent volume.

All values displayed and entries made belong to the current spectrum, selected in the drop down list at the top. The current spectrum displays as a thicker line in the Graph window.

- **Legend Title**: can be changed in the upper drop down list for each spectrum. With *<Up>* and *<Down>* keys you can scroll through the list of Legend Titles, the same works with the dialog's *[<= Back]* and *[Forward=>]* buttons. Of course, spectra can also be directly selected in the upper drop down list. All entries are automatically updated on making changes to the selected spectrum. With *[Replace by filename]* the legend title of the selected spectrum will be replaced by its filename.

With *[Change all titles...]* you can work on all legend titles in a single window. The '#' character may be used as a wildcard with search/replace. There, it is also possible to replace the legend texts by their file names.

- **Line Width:** can be set for any spectrum in relation to the general line width which is changed in menu items *[Thicker lines]* and *[Thinner Lines]*. For exporting as graphics and printing, it is recommended to select line widths from 2 to 5, depending on the outcome of the printed graph.

- **Temperature:** For some spectral file types (*.ggg, *.gdm, *.gkl, *.gpo) the measuring temperature is shown. You may enter your own values.

- **Wavelength:** For some spectral file types (*.sp, *.ggg, *.prn, *.spe) the wavelength is shown (excitation wavelength for fluorescence spectra, detection wavelength for excitation spectra). You may enter your own values. The only commercial file type having it's wavelength inside is *.sp (Perkin Elmer fluorescence spectra).

- **Line Type:** there are three line types possible which can be set for each spectrum separately.

- **Line Colour:** the line colour of the current spectrum is shown in the vertical colour field on the right. There are 14 different colours in the palette which cycle infinitely. The line colour can be set for each spectrum separately with the button *[Change colour =>]*. There you can also define your own colours.

- **Absorption Path Length:** standard value is $d = 1 cm$. Changes have an effect on y axis scaling with ε and $\log(\varepsilon)$, as well as for calculation of concentration via the molar decadic absorption coefficient with the menu item *[Concentration]* (4.9).

- **Concentration:** standard value is **0 mol/l**. For y axis scaling with ε and $\log(\varepsilon)$ a concentration $c \neq 0$ has to be entered. After entering values for molecular weight, sample weigth and solvent volume, the concentration can be calculate with the button *[Calculate]*.

3.15. Menu Item [Information]

For the spectrum selected in the upper drop down list the following properties are shown: filepath, filename, date, description, comment1, comment2, spectrum type, solvent, absorption path length, concentration, wavelength, temperature, start wavelength, end wavelength, step width, number of data points.

The text in the lower part may be copied.

3.16. Menu Item [Normalize]

Normalization in this context means to set the maximum y value of the spectrum to 1.

All spectra are normalized to 1 at their maximum wavelength in the currently displayed x axis range. To select a certain maximum for normalization, zoom into an x axis range where this maximum is the highest. Normalization changes the y values irreversibly and can be done as often as you want. This function is very helpful for direct comparison of spectra.

3.17. Menu Item [Baseline Correction]

For offset-correcting of straight baselines that deviate from zero. Call the function and use the mouse zoom function to select the range which should be set to 0. The mean value of y values in the selected region will be subtracted from the spectrum as a constant value.

 Baseline correction will be done for all spectra at once.

3.18. Menu Item [Advanced Baselining]

For cases, where a simple offset baseline correction doesn't work, this menu function gives advanced possibilities. Select the spectrum for adapting the baseline in the upper drop down list. The treated spectrum will be added to the list of spectra. An interactive live preview helps to efficiently adapt the baseline. Three different baseline types exist currently:

- The **linear** option creates a straight baseline, that can be rotated and shiftet up and down.

- The **adaptive** baseline will be most useful for Raman and FTIR spectra, where a slowly curving baseline has to be subtracted from a spectrum with narrower peaks. The "coarseness" can be varied, and the result can be shifted up and down.

- A **scattering solution** has a baseline continuously rising towards short wavelength values. The increase follows a power law, with an exponent depending on particle sizes. The resulting baseline can be compressed or expanded vertically and shifted up and down.

The following settings are available:

- *[treat all spectra]* subtracts the created baseline from all loaded spectra.

- *[remove original]* removes the original spectrum/ spectra.

- *[keep legend]* lets the original legend text unchanged. If unchecked, " (baseline corrected)" will be added to the legend text(s).

- *[create baseline spectrum]* will produce a spectrum from the baseline used. This can be treated like every spectrum (save, etc.)

3.19. Menu Item [Spike Removal]

When using prolonged exposure times with CCD detectors, artefacts from cosmic radiation are increasing. These are called cosmic spikes.

This function detects spikes and interpolates the spectrum in the range of the spike. All other spectral data points remain totally unchanged. Spectra without spikes remain also unchanged. Broad spikes (> 5 data points, quite rare), are not detected to make sure not to change spectral features.

Select the spectrum to be treated in the upper drop down list. The treated spectrum will be added to the list of spectra.

- *[Remove Original]* deletes the original spectrum.

- *[Treat all Spectra]* for treating all spectra, original spectra will be deleted.

- *[Backward spike Removal]* lets the detection algorithm move over the spectrum from right to left. In this way some more spikes might be detected.

- *[Append "spikes removed"]* appends "spikes removed" to the legend titel of the spectrum.

3.20. Menu Item [Play]

This is a just for fun function. The y values are transformed to tone pitches and played from left to right via the case speaker. The playing speed is 50 nm/second, independent of the resolution of the spectrum.

You might even play real music on selection of appropiate y values and wavelengths.

3.21. Menu Item [Smooth]

For **smoothing** all spectra at once. The algorithm is equivalent to using Savitsky-Golay smoothing of third polynomial order from the "Advanced Smoothing" menu item, however smoothing intensity depends on the resolution of spectra ("interval" parameter will be kept between 6 and 30 points).

Smoothed spectra are added to the list of spectra, the legend titles are appended by "smoothed". Keep this function in mind to be a suspicious feature, it means modifying and glossing over the original data.

3.22. Menu Item [Advanced Smoothing]

For better control of the smoothing behavior. Select the spectrum to be smoothed in the upper drop down list. The treated spectrum will be added to the list of spectra. Three different smoothing types exist currently:

- *[moving average]* smoothes by calculating an average value for every data point from a data range around this point with the selected interval size. A rectangular or triangular shape can be choosen. With the triangular shape, more distant data points are down-weighted.

- *[Savitsky-Golay]* smoothes by calculating a least-squares polynomial interpolation value for every data point. The interval size and the polynomial order can be choosen.

- *[Percentile filter]* smoothes by identifying the selected percentile for the data points contained in the interval size successively for every data point. A lower or upper envelope spectrum results upon choosing the 0% or 100% percentile.

The following settings are also available:

- *[treat all spectra]* smoothes all loaded spectra with the selected parameters.

- *[remove original]* removes the original spectrum/ spectra.

3.23. Menu Item [Peaks & FWHM]

Select a spectrum in the upper drop down list. In the lower part, a **peak table** will be shown. If possible, the corresponding FWHM (**f**ull **w**idth at **h**alf **m**aximum) values will be shown. The peak finding threshold is variable from 1 – 95%. For finding minima, activate the *[ ] inverted* checkbox.

The text in the lower part may be copied with copy&paste by using the mouse or by using the button *[Copy Peak Data]*.

3.24. Menu Item [Integration]

After calling this menu item, use the mouse to zoom into the x axis range to be integrated. Left and right border of the zoom box are the integral boundaries. For each spectrum a separate message window is shown, containing the boundaries, the integral value of the spectrum and the average value within the selected range.

Hint: The **oscillatory strength** will be shown additionally, if the axis types wavenumbers and absorption coefficient are selected. Of course, the value of the oscillatory strength makes sense only for selection of the first electronic transition for integration. The following formula is used to calculate the oscillatory strength f:

$$ f = \frac{4\varepsilon_0 m_e c_0^2 \ln 10}{e^2 N_A} \int_{S_0 \to S_1} \varepsilon(\tilde{\nu})d\tilde{\nu} \quad = \quad 4{,}319 \times 10^{-9} \frac{\text{mol} \cdot \text{cm}^2}{l} \int_{S_0 \to S_1} \varepsilon(\tilde{\nu})d\tilde{\nu} $$

3.25. Menu Item [Cut off Spectrum Part]

For reducing the spectral range (x scale). Select the spectrum to be cut in the middle drop down list and enter the boundaries. The treated spectrum will be added to the list of spectra. The new spectral boundaries will be kept as long as the program runs.

- with "all spectra of one type", you can treat all spectra of a certain type simultaneously.

- *[remove original(s)]* removes the original spectrum/spectra.

- *[prepend "part of:"]* prepends "part of:" to the legend titel of the spectrum/spectra.

3.26. Menu Item [Sort]

For changing the order of loaded spectra.. In the left window, spectra order can be changed manually. Use left mouse button for selection (use SHIFT to select multiple spectra). Insert selected spectra with the right mouse button (above the spectrum you are pointing to). With the option *[automatically]* all spectra can be sorted automatically by three criteria in both directions:

- *<Direction>* can be applied to all spectra (alphabetical and inverse alphabetical available).

- *<Temperature>* only for spectra of our home built spectrometers

- *<Wavelength>* for some spectra of our home built spectrometers and Perkin Elmer *.sp spectra, if measured with a fluorescence spectrometer. For *.spe files, the laser wavelength will be displayed, if they are of Raman type. Up and down direction available.

3.27. Submenu [Remove]

3.27.1. Menu Item [Remove All]

All loaded spectra are deleted. You have to confirm this before deletion takes place actually.

3.27.2. Menu Item [Select for Removal]

For removing arbitrary spectra from the current plot. Select spectra for removing on the left, bring them to the right with the button *[>]*. With *[>>]* you can bring all spectra from left to right at once. The same holds with *[<]* and *[<<]* for the opposite direction.

With the option *"remove all spectra of one type"*, you can remove all spectra of a certain type at once.

3.27.3. Menu Item [Remove Last Spectrum]

The last spectrum from the spectrum list will be removed.

4. The CALCULATIONS Menu

4.1. Menu Item [Addition]

The y values of the two spectra selected in the drop down list will be **added**. *[all spectra]* will execute the operation on all spectra present, taking the spectrum selected in the lower dropdown list for calculation. The new spectrum will be added to the spectra list, its legend titel will be "Addition". *[Keep legend text]* will retain the original legend text for the resulting spectrum. The

new spectrum's start and end wavelength will be determined by the overlapping range of the two selected spectra, the x axis stepwidth is taken from the upper spectrum.

4.2. Menu Item [Subtraction]

The y values of the spectrum selected in the lower drop down list will be **subtraced** from the y values of the spectrum selected in the upper drop down list. *[all spectra]* will execute the operation on all spectra present, taking the spectrum selected in the lower dropdown list for calculation. The new spectrum will be added to the spectra list, its legend titel will be "Subtraction". *[Keep legend text]* will retain the original legend text for the resulting spectrum. The new spectrum's start and end wavelength will be determined by the overlapping range of the two selected spectra, the x axis stepwidth is taken from the upper spectrum.

4.3. Menu Item [Multiplication]

The y values of the two spectra selected in the drop down list will be **multiplicated** by each other. *[all spectra]* will execute the operation on all spectra present, taking the spectrum selected in the lower dropdown list for calculation. The new spectrum will be added to the spectra list, its legend titel will be "Multiplication". *[Keep legend text]* will retain the original legend text for the resulting spectrum. The new spectrum's start and end wavelength will be determined by the overlapping range of the two selected spectra, the x axis stepwidth is taken from the upper spectrum.

4.4. Menu Item [Division]

The y values of the spectrum selected in the upper drop down list will be **divided** by the y values of the spectrum selected in the lower drop down list. *[all spectra]* will execute the operation on all spectra present, taking the spectrum selected in the lower dropdown list for calculation. The new spectrum will be added to the spectra list, its legend titel will be "Division". *[Keep legend text]* will retain the original legend text for the resulting spectrum. The new spectrum's start and end wavelength will be determined by the overlapping range of the two selected spectra, the x axis stepwidth is taken from the upper spectrum.

4.5. Menu Item [Spectra Averaging]

The y values of the selected spectra (left mouse button) will be **averaged**. The new spectrum will be added to the spectra list, the legend title can be set in the lower edit field.

4.6. Menu Item [Y Constants]

Apply one of four calculation types to the spectrum selected in the upper drop down list. The new spectrum will be added to the spectra list. The following options are available:

- with *"all spectra of one type"*, you can treat all spectra of a certain type simultaneously.

- *[remove original(s)]* removes the original spectrum/spectra.

- *[append "+ | − | × | ÷ | n |"]* appends calculation type and figure to the legend titel of the new spectrum/spectra.

Hint: For multiplication with λ^2 resp. λ^4 enter **x2** resp. **x4** into the edit field. This feature is helpful when you have to display fluorescence spectra in wavenumbers. Generally you can multiplicate with λ^n by entering **xn** (n: real number). The other calculation types are also applicable.

4.7. Menu Item [X Constants]

Depending on the selected calculation type, you can move spectra to left/right, stretch and compress along the x axis. This function works only if the spectrum's original x axis type is identical with the currently displayed x axis type in the graph window. The new spectrum will be added to the spectra list, its legend title appended by the calculation type and the figure from the edit field.

Hint: Please use this function only if you really know what you are doing, since it modifies the spectra in a rather unusual manner.

4.8. Menu Item [Derivative]

The first, second, third or fourth derivative of the spectrum selected in the drop down list will be calculated. The new spectrum will be added to the spectra list, its legend title appended by "x. Derivative". The following options are available:

- with *"derive all spectra"*, you can treat all spectra simultaneously.

- *[remove original(s)]* removes the original spectrum/spectra.

- *[smooth before derivation]* uses a smoothed spectrum for the derivative function. This feature virtually eliminates the influence of measurement noise.

Hint: Even higher order derivatives can be obtained by repeated derivation of derived spectra. The smoothing option is strongly recommended for all derivatives higher than the first derivative.

4.9. Menu Item [Transmittance/Reflectance]

From two intensity spectra (sample and background/reference), the transmittance or reflectance will be calculated after $T = I/I_0$.

4.10. Menu Item [Degree of Polarisation]

When processing polarization dependent fluorescence and excitation spectra, it is often necessary to calculate the **degree of polarisation** and a **reduced spectrum**.

This can be done from two spectra with different polarizer orientations according to the following equations:

$$P = \frac{I_y - I_x}{I_y + I_x} \qquad (1)$$

$$G = I_y + 2 \cdot I_x \qquad (2)$$

With: G : **reduced spectrum**

        P : **degree of polarisation**

        I_x : fluorescence intensity with crossed polarizers

        I_y : fluorescence intensity with parallel polarizers.

The two spectra are selected in the two drop down lists. The new spectrum will be added to the spectra list, the legend title of the new spectrum consists of the legend title of the spectrum and the new spectral type.

To calculate all spectra at once, use the option *[all]*. This works only for spectra of *.ggg type.

4.11. Menu Item [Raman Shift]

Here you can calculate a Raman shift spectrum from a (fluorescence) intensity spectrum in the wavelength scale and by providing the laser excitation wavelength. As the area around 0 cm^{-1} is usually distorted due to the laser emission itself, you can define the start wavenumber of the Raman spectrum. Two more options are available:

- *[transform all spectra]* applies the transformation to all loaded spectra at once.

- *[remove original(s)]* removes the original wavelength spectrum/ spectra from display.

4.12. Menu Item [Concentration]

In addition to the menu item *[General]* (3.14), this is the second possibility to set the concentration for a certain spectrum. For this function, the molar absorption coefficient has to be known. The calculation of concentration is done via **Lambert-Beer** (Equation(6)).

All entered values apply only to the spectrum selected in the upper drop down list. Upon entry of molar absorption coefficient, wavelength and absorption path length, you might calculate the concentration with the button *[Calculate]*. You can enter the maximum of the current spectrum automatically with the button *[Insert Peak]*.

Hint: For directly entering the concentration, use the menu item *[General]* (3.14).

4.13. Menu Item [Effective Absorbance]

For the determination of fluorescence quantum yields it is important to know the fraction of light absorbed by the sample. Due to the finite bandwith of the excitation light, this has to be averaged across the bandwidth range, yielding the "effective absorbance".

The following formula is used:

$$A = \frac{\int A(\lambda)I(\lambda)d\lambda}{\int I(\lambda)d\lambda} \tag{3}$$

with $A(\lambda)$: absorption spectrum

         $I(\lambda)$: bandwidth of excitation light

Normally, the bandpass is a triangular function characterized by its center wavelength and bandwidth.

4.14. Menu Item [Center of Gravity]

For the spectrum selected in the drop down list, the **center of gravity** will be calculated. I. e. the mean of x values $\langle\lambda\rangle$ resp. $\langle\tilde{v}\rangle$ weighted by the y values (absorbances $A(\lambda)$ resp. $A(\tilde{v})$) after the following equation:

$$\langle\lambda\rangle = \frac{\int \lambda A(\lambda)d\lambda}{\int A(\lambda)d\lambda} \quad \text{resp.} \quad \langle\tilde{v}\rangle = \frac{\int \tilde{v}A(\tilde{v})d\tilde{v}}{\int A(\tilde{v})d\tilde{v}} \tag{4}$$

The current x axis range will be used as integration boundaries. The result will be shown in the middle part of the status bar.

Hint: the center of gravity in wavelength scale does not correspond to the reciprocal center of gravity in wavenumbers scale: $\langle\lambda\rangle \neq \langle\tilde{v}\rangle^{-1}$!

4.15. Menu Item [Film Thickness]

From a white-light reflectance spectrum spectrum showing interference, the thickness of a single-layer thin film can be calculated. As input values, the refractive index of the film material is needed together with the incident angle of the reflected light (perpendicular illumination means an angle of 0°). The wavelength range used can be changed.

The results window contains the input data, the number of evaluated peaks and the calculated thickness together with a quality measure (StDev). A StDev value higher than a few percent of the thickness value indicates an invalid result.

Hint: This is a new and experimental feature; user feedback is strongly needed and important for the future development of this feature! Do not use this method without cross-checking the results by an alternative method!

4.16. Menu Item [Quantum Yield]

This menu item is too complicated to explain here. Ask me directly for explanations.

4.17. Menu Item [Low Temp. Quantum Yield]

A useful explanation would go beyond the scope of this manual.

5. The Fluorescence/EEM Menu

5.1. Menu Item [Emission Sensitivity Correction]

For correction of the wavelength dependent sensitivity of detectors of fluorescence spectrometers. This function yields "**true spectra**".

 Load the correction curve with the button *[Load correction Curve]* (*.fak file, has to consist of XY pairs with sensitivity against wavelength). Select the spectrum to be corrected in the middle drop down list. The corrected spectrum will be added to the list of spectra, the legend title will be appended with "corrected".

- *[remove original]* removes the original spectrum.
- *[correct all]* corrects all loaded spectra and deletes the original spectra.

5.2. Menu Item [Excitation Wavelength Assignment]

For useful handling of a set of fluorescence spectra as two-dimensional spectrum or EEM (**e**xcitation **e**mission **m**atrix), each spectrum needs its excitation wavelength assigned. This can be entered manually for each spectrum in the menu item *[General]* (3.14), or automated for a complete set of spectra from this menu item. Just enter the excitation wavelength of the first spectrum and the excitation step width. In case your EEM set comprises only a part of the loaded spectra, select the first and last spectrum of the set in the two drop down lists. For naming the spectras' legend texts with the excitation wavelength, activate the "replace spectrum names…" checkbox.

5.3. Menu Item [Correction of Excitation Intensity]

For correcting the wavelength dependent excitation intensity (lamp spectrum) of fluorescence spectrometers for a series of fluorescence spectra with known excitation wavelength.

Load the lamp correction curve with the button *[Load Lamp Spectrum]*. Select the range of spectra to be corrected with the two drop down lists. The corrected spectra will replace the original spectra, their legend titles will be appended with "Excitation intensity corrected".

Hint: The wavelength can be entered manually for each spectrum in the menu item *[General]*.

5.4. Menu Item [Remove Rayleigh/ Raman Scatter]

Often enough, and especially for samples with low intensity fluorescence, EEMs are distorted by scattering peaks. These come from first and second order Rayleigh scattering as well as Raman scattering and can't be fully suppressed even by choosing optimal geometries of sample container and optical pathways. For being further used in chemometrics analysis, scattering has to be removed. Traditionally this has been done by setting the scattering regions to zero, but there are much better ways to reduce the influence of straylight.

This menu item allows to remove all scattering from a whole EEM set at once with this options:

- *[treat all spectra]* applies the treatment to all loaded spectra at once.

- *[remove original(s)]* removes the original spectrum/ spectra from display.

- With *[replace by]* you can replace the values of the scattering region by

    o **zeroes**, this is the traditional & most ugly way of doing it

    o a **straight line**, which might be better in some cases

    o an **interpolated curve**, which is most useful, because it give the least distortion. The interpolated values have the same noise level as their surrounding

- set the excitation **bandwith** of your spectrometer, this defines the width of the replaced values area

- [remove Rayleigh peaks (1st order)] removes the scattering intensity around your excitation wavelengths (visible because your sample does it)

- [remove Rayleigh peaks (2nd order)] removes the scattering intensity around twice your excitation wavelengths (visible because your emission monochromator leaks the 1st order scattered light into it's 2nd order wavelength range)

- [remove Raman peaks (1st order)] removes the scattering intensity around the Raman-shifted excitation wavelengths. The wavelength difference is solvent dependent, some solvents can be selected from the drop down list. You can include other solvents by editing the file "Raman-Bands.csv" from the Spekwin32 program folder.

5.5. Menu Item [Subtract Blank EEM dataset]

It might be useful to subtract the fluorescent properties of a "Blank" sample from the EEM you want to analyze. This can be successfully used to remove background fluorescence and also scattering peaks.

In the upper part you can see the dimensions of your current EEM set. Load the Blank dataset to be subtracted with *[Load Blank dataset…]*. This naturally will only work if the dimensions of both datasets are identical. Optionally, "blank subtracted" can be appended to the legend texts.

5.6. Menu Item [Show 2D Spectrum (EEM)]

For two-dimensional display of a series of fluorescence or excitation spectra. EEM means: **E**xcitation **E**mission **M**atrix. The spectra should be connected to a wavelength and be corrected for excitation intensity before (see above).

The current mouse position is shown in the lower left corner as excitation, emission and intensity values. On the right there are some options for display. By default, excitation is oriented horizontally, emission goes vertically. This orientation can be exchanged by using the [switch x <=> y axes] option. The EEM plot can be copied to clipboard with [Copy => Clipboard].

You cannot print or save the graph at the moment, there are no axes.

5.7. Menu Item [Calculate Excitation Spectra]

For calculating a series of excitation spectra from a series of fluorescence spectra. The spectra have to be connected to a wavelength and should be corrected for excitation intensity before (see above).

Enter the range and stepwidth of detection wavelengths for the excitation spectra. Fluorescence spectra will be removed if *[Remove Fluorescence spectra]* is checked.

6. The GaussFit Menu

These functions have been taken over almost unchanged from the 16bit version called Spekwin. They are working, but not unimpeachable.

For simulating spectra with Gauss-type groups of curves and fitting of real spectra. Will work with wavenumbers only as x axis type.

6.1. Menu Item [Manually]

Here the real work is done. A guidance document is available from the Spekwin32 website.

6.2. Menu Item [Open]

To open a previously saved dataset of Gauss spectra (*.gss).

6.3. Menu Item [Save]

To save the current dataset of Gauss spectra.

7. The PLOT/OPTIONS Menu

7.1. Menu Item [Configuration]

Here you can change and set permanently many program options. The settings are written to the file spekwin32.ini in the program folder. Write access is needed for this file! During program start, these settings are read from the file. Without write access, the settings can be read but not written. It is recommended to install Spekwin32 to a folder with write access.

With [allow multiple instances] activated, it is not advised to load many spectra at once by double clicking within the file explorer. This will open as many Spekwin32 windows as files were selected, which might slow down system performance in an unpleasant way☺

7.2. Menu Item [Show Peak Labels]

Activation of this menu item will bring up an additional menu bar for all the options connected with the automated peak labeling feature.

- *Show label* allows to use x or y values or both combined as peak label.

- *Threshold* defines the mimimum value for labeled peaks. It's a percental threshold, calcualated for each spectrum individually.

- *Prominence* is a parameter that allows fine-tuning the selection of peaks for labeling. Works similar to the "topographic prominence" parameter used to describe mountain tops.

- With *Digits*, you can adjust the number precision of the displayed values.

- The *angle* of the label text can be varied between all angles from vertical to horizontal.

- You can either label peaks for *all spectra* or *select* a single *one*.

<u>Hint:</u> There is no way to individually label peaks!

7.3. Menu Item [Show Spectroscope View]

The "Spectroscope View" tries to reproduce the visual appearance of the measured sample viewed through a hand spectroscope. These are still in wide use in some industries, especially with gemologists. It might provide helpful to reduce the gap between visual and spectrometric

methods. This feature works for absorbance or transmittance spectra as well as for emission spectra.

Activation of this menu item will show an additional menu bar below the plot for all the options connected to the Spectroscope View. A fully blown "rainbow spectrum" is shown, with reduced brightness according to the selected spectrum's absorbing regions.

- Within the rainbow spectrum, the wavelength scale can deactivated and the *tick* distance changed.

- The coloured rainbow spectrum can be shown as *grayscale* representation (For those with black&white screens and colourless textbooks ☺)

- *[Align]* causes the upper spectrum plot to be aligned with the lower rainbow spectrum. Depending on monitor and window size, this may not always work perfectly…

- With *[activate]*, the modulation of the full rainbow spectrum by the measured spectrum can be switched on and off. Select the spectrum with the drop down list.

- With *[CopyToClipboard]* you can place the current rainbow spectrum into the Clipboard.

- *[Save as..]* will save the current rainbow spectrum to the hard disk.

Hint: Transmittance values above 1 (which make no sense physically) lead to distorted colours. Emission spectra should be normalized or kept below 1 for y values, otherwise colors will also be distorted.

7.4. Menu Item [Axes]

For interconversion of different axis types and naming of axis titles. For the x axis, **wavelength** and **wavenumbers** scale can be selected. Wavenumbers and electron volts are proportional to energy. The following equation holds true if the axis units are nm and 1/cm:

$$\lambda = \frac{10^7}{\tilde{\nu}} \quad \text{und} \quad \tilde{\nu} = \frac{10^7}{\lambda} \tag{5}$$

Additionally, axis conversion to **electron volts** (eV) is possible. The calculation is: 1eV=8065,5cm-1. There is an option to manually set the x axis boundaries in the *[boundaries]* window.

The y axis values can be shown as transmittance T, absorbance A, molar absorption coefficient ε and log(absorption coefficient) log ε. For interconversion, the following equations are used:

$$A = -\log T \quad \text{and} \quad A = \varepsilon c d \tag{6}$$

For displaying epsilon and log epsilon you have to assign a concentration to the spectrum. This can be done in the menu items *[General]* (3.14) or *[Concentration]* (4.12). Additionally, the y type "Reflectance" and "Intensity" are recognized and handled by Spekwin32.

If possible, the x and y axis types are choosen automatically according to the most recently opened spectral file.

The display of axis titles can be turned off with the option [show Axis Title]. Set your own individual axis title with [Set Axis Title].

The option *[Alternating Tick Label]* is enabled by default when set in the menu item *[Configuration]*.

7.5. Menu Item [Axes Font]

For changing the font of both axes' tick labels.

7.6. Menu Item [Legend Font]

For changing the font of both axis titles and of legend titles.

Hint: When choosing very big font sizes, the axes titles and axes labels might overlap.

7.7. Menu Item [Legend on/off]

For turning on and off the display of legend titles.

7.8. Menu Item [Grid on/off]

For turning on and off the grid display.

7.9. Menu Item [Secondary x-Axis on/off]

For turning on and off the complementary second x axis at the upper edge of the graph window.

7.10. Menu Item [Points/Lines]

For switching spectral display as points or lines.

7.11. Menu Item [Flip X-Axis]

For switching x-Axis direction. Will affect only the graphics display. Data will be saved in default direction.

7.12. Menu Item [Standard Lines&Colours]

For setting back spectral display to default values. The colours are set to default order, line width to 1 and line style to "solid".

7.13. Menu Item [Thicker Lines]

Increase the line width by one. Possible range is from 1 to 7.

7.14. Menu Item [Thinner Lines]

Decrease the line width by one. Possible range is from 1 to 7.

7.15. Menu Item [Plot All Black]

Set all spectra's line colour to black. To go back, call the menu item *[Standard Lines & Colours]* once.

7.16. Menu Item [Cycling Line Types]

Sets the line types of all spectra to alternate between "solid", "dotted" and "dashed". To go back, call the menu item *[Standard Lines & Colours]* once.

7.17. Menu Item [Display Values]

Continuously shows the current x and y value for the spectrum selected in the drop down list. Close with *[Abort]*.

7.18. Menu Item [Background Colour]

For changing the graph's background colour. The background colour will not be plotted, exported or saved, the background remains transparent!